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Taxol and tau overexpression induced calpain-dependent degradation of the microtubule-destabilizing protein SCG10

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Abstract

Microtubule-stabilizing and -destabilizing proteins play a crucial role in regulating the dynamic instability of microtubules during neuronal development and synaptic transmission. The microtubule-destabilizing protein SCG10 is a neuron-specific protein implicated in neurite outgrowth. The SCG10 protein is significantly reduced in mature neurons, suggesting that its expression is developmentally regulated. In contrast, the microtubule-stabilizing protein tau is expressed in mature neurons and its function is essential for the maintenance of neuronal polarity and neuronal survival. Thus, the establishment and maintenance of neuronal polarity may down-regulate the protein level/function of SCG10. In this report, we show that treatment of PC12 cells and neuroblastoma cells with the microtubule-stabilizing drug Taxol induced a rapid degradation of the SCG10 protein. Consistently, overexpression of tau protein in neuroblastoma cells also induced a reduction in SCG10 protein levels. Calpain inhibitor MDL-28170, but not caspase inhibitors, blocked a significant decrease in SCG10 protein levels. Collectively, these results indicate that tau overexpression and Taxol treatment induced a calpain-dependent degradation of the microtubule-destabilizing protein SCG10. The results provide evidence for the existence of an intracellular mechanism involved in the regulation of SCG10 upon microtubule stabilization.

Keywords: SCG10; Tau; Taxol; Microtubules; Degradation; Calpain

Introduction

The elongation and rapid shortening of microtubules, a process referred to as dynamic instability, is crucial during neuronal differentiation (Mitchison and Kirschner, 1984; Dent and Gertler, 2003). This process responds to external cues that control and guide molecular changes required for the establishment of domains necessary for neuronal function (Dent and Gertler, 2003). Several proteins that affect microtubule's dynamic instability during neuronal differentiation have been identified (Dent and Gertler, 2003). Some of these proteins, such as SCG10 and stathmin, destabilize microtubules whereas others like the microtubule-associated protein tau promote microtubule stabilization (Riederer et al., 1997; Buèe et al., 2000; Grenningloh et al., 2004). Several studies indicated that both microtubule-stabilizing and -destabilizing proteins are essential for neurite outgrowth and neuronal function (Buèe et al., 2000; Grenningloh et al., 2004). However, it is still unknown whether these functional diverse proteins can affect or regulate each other's functions to coordinate microtubule's dynamic instability.

SCG10 is a neuronal-specific protein of the same gene family as stathmin (Grenningloh et al., 2004). The level of SCG10 protein is elevated during development and reduced in adults, suggesting that its expression is developmentally regulated. This protein is found to associate with membrane rafts and Golgi (Lutjens et al., 2000; Maekawa et al., 2001). Additionally, SCG10 is enriched in growth cones during neurite outgrowth (Lutjens et al., 2000; Grenningloh et al., 2004). Analysis of purified SCG10 protein from brain protein extract detected the presence of

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higher molecular weight SCG10 species (Antonsson et al., 1998). Further studies demonstrated that this shift in molecular weight is due to phosphorylation. In vitro studies have shown that protein kinases A (PKA) phosphorylates SCG10 at Ser50 and Ser97, whereas mitogen-activated protein kinase (MAPK) does it at Ser62 and Ser73 (Antonsson et al., 1998; Grenningloh et al., 2004). Furthermore, cyclin-dependent kinases, such as CDK5, have been shown to phosphorylate SCG10 at Ser73 (Grenningloh et al., 2004). These phosphorylation events adversely affect the microtubule-destabilizing activity of SCG10 protein (Antonsson et al., 1998; Grenningloh et al., 2004).

The microtubule-associated tau proteins are preferentially expressed in neurons (Buèe et al., 2000). However, tau expression has been also detected in other cell types (Buèe et al., 2000). Similarly to SCG10, the biological function of tau proteins is adversely affected by phosphorylation (Buèe et al., 2000; Lee et al., 2001). Hyperphosphorylation of tau proteins and its aberrant intracellular aggregation have been identified as a hallmark in a family of neurodegenerative disorders, collectively known as tauopathies (Lee et al., 2001). The best known and studied tauopathy is Alzheimer's disease (AD). The pathological role of tau in these disorders is underscored by the identification of mutations in the tau gene of kindred afflicted by neurodegeneration (Lee et al., 2001). Most of the identified mutations interfered with the ability of tau proteins to bind tubulin and stabilize microtubules (Buèe et al., 2000; Lee et al., 2001). Based on these findings, a loss-of-function model has been suggested to explain tau-induced neurodegeneration (Feinstein and Wilson, 2005). Consistently, a tauopathy mouse model treated with low dosages of the microtubule-stabilizing drug Taxol exhibited a restoration of fast axonal transport, increase axonal microtubules, and improved motor impairments (Zhang et al., 2005). Additionally, Taxol protected primary neurons from the toxic effect induced by β-amyloid peptides ($A\beta_{1-42}$) and mitigated tau phosphorylation (Michaelis et al., 2004). These results suggest that microtubule depolymerization due to disruption of the biological function of tau may play a fundamental role in the molecular mechanism underlying tau-induced neurodegeneration.

Regulation of the biological function of microtubule-stabilizing and -destabilizing proteins may be inherently interconnected. Here, we showed that both Taxol treatment and tau overexpression induced a drastic reduction in SCG10 protein level. Calpain inhibitor but not proteasomal or caspase inhibitors blocked this reduction in SCG10 protein. Collectively, these results suggest that microtubule stabilization triggers a signaling cascade that leads to the activation of calpains and degradation of microtubule-destabilizing proteins.

Materials and methods

Materials

Antibodies used in this project are polyclonal antibodies WK S44 specific to human tau (aa 162–178; 1:500; generated by Dr. Yen's laboratory; DeTure et al., 2002), SCG10-BR (Antonsson et al., 1998), SCG10-GL (DiPaolo et al., 1997), anti-actin (C-11; Santa Cruz Biotechnology), and anti-cleaved caspase-3 (Asp175; Cell Signaling Technology). Monoclonal antibodies used are anti-α-tubulin and anti-ubiquitin (P4D1) from Santa Cruz Biotechnology. Secondary antibodies used in western blot analysis are peroxidase-conjugated goat anti-rabbit (1:4000) or goat anti-mouse (1:2000) Ig antibodies (Chemicon, Temecula, CA, USA).

Pharmacological agents used are described below. Taxol (Paclitaxel) and Nocodazole were purchased from Sigma. All other drugs, including epoxomicin, MG132, calpain inhibitor III (MDL-28170), caspase inhibitor I (Z-VAD-FMK), lactacystin, H-89 dihydrochloride, roscovitine, MEK inhibitor (PD 98059), and JNK inhibitor II (SP600125) were purchased from EMD Biosciences. The specific concentrations used are described in the text.

Culture and drug treatment of PC12 cells

Pheochromocytoma cells (PC12) were maintained in growth media [DMEM supplemented with 7.5% fetal calf serum (FCS), 7.5% horse serum (HS), 3.5 g/L glucose, 3.7 g/L sodium bicarbonate, pH 7.3, 10 μg/mL streptomycin, and 10 U/mL penicillin] at 37°C/5%CO_2. Cultured undifferentiated PC12 (5×10^6 cells/plate; five plates per treatment) were treated with different concentrations of Taxol (dissolved in ethanol) for 24 h. As control, equal volume of ethanol was added to culture PC12 cells for the same interval of time. When indicated, PC12 cells were pre-treated with specific inhibitors, at the concentration specified in the text, for 3 h before the addition of 10 μM Taxol to the medium. To induce differentiation, 100 ng/mL of nerve growth factor (NGF) was added directly to PC12 cells cultured in minimal medium [DMEM supplemented with 3.5 g/L glucose, 3.7 g/L sodium bicarbonate, pH 7.3, 1% FCS, 1% HS, 10 μg/mL streptomycin, and 10 U/mL penicillin] at 37°C/5%CO_2. PC12 cells were incubated in NGF containing media for three days prior to Taxol (10 μM) treatment for 24 h NGF-differentiated PC12 cells (for 3 days) were pre-treated with calpain inhibitor (MDL28170) for 3 h prior to the addition of Taxol (10 μM) to the medium.

Neuroblastoma cells and tau overexpression

The Tet-off inducible system was used to generate stable transfectant (M1C) expressing wild-type human tau (4R0N). M1C cells were derived from human neuroblastoma BE(2)-M17D cell line and maintained in growth media [DMEM supplemented with 10% fetal bovine serum (FBS), 4.5 g/L of glucose, 3.7 g/L sodium bicarbonate, pH 7.3, 0.3 g L-glutamine, 400 μg/mL G418, 1 μg/mL puromycin, 2 μg/mL tetracycline (Tet), 100 μg/mL zeocin, and 100 μg/mL hygromycin] at 37°C/5% CO2. The cells were seeded in 10 cm dish at 2×10^6 cells per plate (60–70% confluence) a day before Tet-off induction. Tau expression was induced by thoroughly washing the cells with DMEM (without Tet) and adding fresh medium with different concentrations (1 ng/ml, 10 ng/ml, or 100 ng/ml) of Tet. Cells were cultured in the indicated medium for seven days. Fresh medium was supplemented on the third and fifth day. When indicated, M1C cells were pre-treated with calpain inhibitor MDL28170 (10 μM) or proteasome inhibitor MG132 (1.0 μM or